



Anthelmintic potency of garlic (*Allium sativum*) and neem (*Azadirachta indica*) combination as herbal anthelmintic in lactating Karan Fries cows

MAMTA¹, S S LATHWAL², PAWAN SINGH², INDU DEVI^{3✉} and AJAY KUMAR¹

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

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Gastro-intestinal parasites affect the health and productivity of dairy cows, and cause considerable cumulative economic losses to farmers (Iqbal *et al.* 1993). If remain untreated, they are responsible for causing nearly one-third of total losses due to all animal diseases (Anonymous 1990). Even sub-clinical infection of parasites can result in reduced feed intake and decreased growth in cattle (Szyszka *et al.* 2013). Nematodes have been found as the major helminth problem (40-60%). The strongyles are the maximum prevalent nematodes of cattle and their infections showed highest prevalence during rainy season, followed by winter and summer (Renwal *et al.* 2017). A number of studies reported the economic losses attributed due to parasitic load in form of reduced work capacity, lower fertility, reduction in food intake, lower weight gains and milk production, treatment costs, involuntary culling, higher susceptibility of animals to secondary infections due to lowering down of the immune status and mortality in heavily parasitized animals (Rinaldi *et al.* 2011). To deal with this problem of gastrointestinal parasitism, when it comes to treatment, either they are commonly overlooked, as majority of the infected animals show little obvious clinical signs and their effects are gradual and chronic (Raza *et al.* 2010), thus not drawing the immediate attention of the farmer, or treated conventionally with commercially available chemical anthelmintics. However, these are very expensive and sometimes unavailable to farmers in rural areas, and also have some associated adverse effects. Furthermore, regular use of chemical anthelmintics develops resistance of helminths to various anthelmintic compounds. Emergence of anthelmintic resistance along with associated adverse effects and the high cost of synthetic anthelmintics, create the need of alternative approaches. Herbal based anthelmintics could be the best possible alternative of these synthetic anthelmintics, as

plants has long been used for ages by farmers and traditional healers to treat parasitism and improve performance of livestock. The World Health Organization has estimated that 80% of the populations of developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs (WHO 2008). Regardless of their wide ethnoveterinary usage, scientific evidence on the anti-parasitic efficacy of most plant products is limited. Many *in vitro* studies have been conducted to demonstrate the anthelmintic efficacy of different herbs, some of them reported neem and garlic as the most efficacious for *in vitro* anthelmintic activity (Amin 2009).

Neem (*Azadirachta indica*) has biologically active component azadirachtin, which is having antifungal and anti-microbial properties (Kudom *et al.* 2011, Ramesh *et al.* 2011). The anthelmintic property of neem is due to azadirachtin (active alkaloid), which inhibits the cholinergic transmission in CNS of parasite and partly blocks calcium channel thereby resulting in expulsion of parasite from the animal body (Qiao *et al.* 2014). Garlic (*Allium sativum*) has amebicide, parasiticide, acarifuge, vermifuge, larvicide, antifungal and immuno-stimulants as well as anti-helmintic properties (Duke 2002). These two herbs are widely distributed and also known in every household for their wide range of uses. However, *in vivo* studies need to be conducted for practical application of *in vitro* results on their anthelmintic efficiency or success. Considering the aforementioned facts, this study was conducted to evaluate *in vivo* anthelmintic efficacy of garlic and neem combination in Karan-Fries cows from 2019 to 2020.

Neem leaf powder was purchased from local market after assessing their quality with help of Ayurvedic practitioner and drug manufacturer. Garlic bulbs were also procured from the local market and dried under the shade for 15 days. After drying and removal of outer husks, the bulbs were grinded to fine powder with the help of electrical mixer. The herbal mixture was prepared after mixing pulverized herbs in specific proportion. The dose was calculated for the respective animal based on their body weight, then garlic and neem powder were weighed accordingly in the ratio of 1:5 (based on the dose rate obtained by the *in vitro*

Present address: ¹Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, Uttar Pradesh. ²ICAR-National Dairy Research Institute, Karnal, Haryana. ³ICAR-Central Institute for Research on Cattle, Meerut, Uttar Pradesh. ✉Corresponding author email: indulathwal@gmail.com

standardization). Twenty-eight freshly calved crossbred Karan Fries (Tharparkar × Holstein Friesian) cows, in first to third parity with most probable production ability (MPPA) of around 3312.13±87.18 kg milk, were selected. These cows were dewormed at least six months before the start of the experiment. The animals were kept under loose housing type system. They were fed with *ad lib.* green fodder and measured amount of concentrate diet (20% CP and 70% TDN) as per NRC (2001). Fresh and clean drinking water was available to animals (*ad lib.*). Animals were selected around 15 days before expected date of parturition and given adaptation period of 7 days. They were randomly divided into four groups, viz. control (T1), synthetic dewormer (T2), single dose of herbal anthelmintic (T3) and double dose of herbal anthelmintic (T4). In control group (T1), the routine feeding practices of animals were followed without any deworming. Animals in treatment groups were fed the same ration as in control group but provided with dewormer. In T2 group, the synthetic dewormer Macfen-plus™ (combination of Ivermectin and Fenbendazole) was used. In T3 group, garlic+neem in the ratio 1:5 was given @ 4 g/kg body weight as single dose on day 7 after calving. In T4 group, in addition to single dose on day 7 after calving, same dose was again repeated on day 60. The experiment was started 7 days post calving and continued upto 120 days. Sampling for fecal sample was done on 0, 1, 2, 3, 4, 5, 6, 7, 8 fortnights relative to the deworming treatment. For *in vivo* efficacy of the herb,

fecal evaluation was done for parasitic eggs. Fecal samples were collected directly from the rectum, with disposable glove, at fortnight interval and evaluated for egg per gram by McMaster technique. Faecal egg count reduction was calculated as:

$$\text{FECR}\% = 1 - (\text{Xt}/\text{Xc}) \times 100 \quad (\text{Coles } et al. 1992)$$

where t, post treatment EPG; c, pre treatment EPG.

Body weight of the animals was recorded at fortnight intervals. The animals were weighed for two consecutive days in the morning before offering feed and water. The average body weight of two days was considered as body weight for that fortnight. The feed efficiency (FE) was calculated for milk yield by dividing average milk yield (kg/day) by average DMI (kg/day) for individual animal. Body condition score (BCS) of experimental cows was assessed by visual inspection and palpation once every fortnight. The scoring was done by the same person throughout the study period. The data obtained in the present study was analyzed by one way ANOVA and the significance of the differences between the mean values of various parameters were determined by Duncan's Multiple Range test using SPSS (version 20.0). All the data is presented as mean±SE. Statistical significance was taken at the 5% level of significance ($P < 0.05$).

The changes in the egg per gram count (EPG) and faecal egg count reduction (FECR%) of different groups for 8 fortnights is presented in Tables 1 and 2 respectively. After

Table 1. Mean±SE of faecal egg counts (egg/g) of cows

Fortnights	Control (T1)	Synthetic (T2)	Herbal single dose (T3)	Herbal double dose (T4)
0	714.29±96.19	785.71±70.47	814.29±50.84	728.57±68.21
1	800.00±75.59	28.57±18.44	328.57 ^b ±71.43	300.00 ^b ±78.68
2	814.29 ^b ±67.01	0.00±0.00	157.14 ^a ±57.14	128.57±60.61
3	771.43±74.69	0.00±0.00	185.71 ^b ±26.08	185.71 ^b ±26.08
4	742.86 ^c ±68.51	85.71 ^a ±26.08	300.00 ^b ±57.74	242.86 ^b ±36.89
5	714.29±88.45	85.71 ^a ±14.29	242.86 ^b ±48.09	42.86 ^a ±20.20
6	728.57 ^d ±56.54	285.71 ^b ±59.48	500.00 ^c ±37.80	85.71 ^a ±26.08
7	800.00 ^d ±53.45	285.71 ^b ±59.48	542.86 ^c ±61.17	57.14 ^a ±20.20
8	857.14 ^d ±1.90	228.57 ^b ±56.54	500.00 ^c ±57.74	57.14 ^a ±20.20
Overall	771.43 ^y ±23.6	196.83 ^w ±32.16	406.35 ^x ±32.94	195.24 ^w ±28.03

Means bearing different superscript in a row differ significantly ($P < 0.05$).

Table 2. Mean±SE of faecal egg counts reduction (FECR %) of cows

Fortnights	Control (T1)	Synthetic (T2)	Herbal single dose (T3)	Herbal double dose (T4)
1	-17.86 ^a ±10.89	96.79 ^c ±2.09	57.36 ^b ±11.44	60.24 ^b ±7.21
2	-19.02 ^a ±8.10	100.00 ^c ±0.00	78.57 ^b ±9.01	85.00 ^b ±7.13
3	-13.89 ^a ±12.94	100.00 ^c ±0.00	75.64 ^b ±4.96	68.69 ^b ±7.70
4	-8.12 ^a ±7.59	87.98 ^c ±3.61	61.73 ^b ±9.01	55.36 ^b ±14.44
5	-2.99 ^a ±10.72	88.93 ^c ±2.24	70.66 ^b ±4.92	95.00 ^c ±2.38
6	-6.90 ^a ±8.45	62.26 ^c ±8.98	37.97 ^b ±4.25	85.24 ^d ±4.69
7	-18.63 ^a ±10.03	62.02 ^c ±8.78	33.42 ^b ±6.20	93.21 ^d ±2.42
8	-25.03 ^a ±7.57	69.17 ^c ±8.04	37.61 ^b ±7.53	88.10 ^d ±5.02
Overall	-14.06 ^w ±3.34	83.39 ^y ±2.76	56.62 ^x ±3.39	78.85 ^y ±3.09

Means bearing different superscript in a row differ significantly ($P < 0.05$).

first fortnight, there was a significant reduction in EPG count in all the dewormer treated groups compared to the control group, where there was rather increase in FECR ($-17.86 \pm 10.89\%$). In all the anthelmintic treated groups, mean EPG values were significantly ($P < 0.05$) lower compared to the control group. In synthetic dewormer group (T2), significantly highest reduction than the herbal treated groups T3 and T4, was observed. The overall mean values of FECR also showed an increase ($-14.06 \pm 3.34\%$) in the egg count in the control group, whereas decrease in egg count was observed in all the treated groups. The synthetic dewormer treated group (T2) and the double dose herbal treated group (T4) did not show significant difference in the overall FECR although it was significantly better than the single dose in T3 group.

The high faecal egg counts in the control group could be attributed to the high presence of adult parasites in reproductive stages in the host (Worku *et al.* 2009). The decrease in faecal egg counts in all the anthelmintic treated groups from first to eighth fortnight, indicated that garlic and neem as herbal anthelmintic had a positive effect on the GI nematodes. The significant reduction after fourth fortnight in T4 group might be due to the second dose effect. The presence of alkaloids, glycosides and tannins have been suggested as the responsible chemical constituents for anthelmintic activity of garlic and neem. The efficacy of chemical dewormer (96% at first fortnight and 100% by the second fortnight) was found in corroboration with many previous studies. A study reported 98.80% efficacy of ivermectin and 100% efficacy of fenbendazole on eighth day respectively, post treatment against *Strongyle* worms (Tramboo *et al.* 2017). Another study reported 100% efficacy of ivermectin and 98.80% of fenbendazole on 14th day post treatment. T4 group showed maximum reduction in EPG by second fortnight (about 80%). Analogous findings were reported by previous studies. Jamara *et al.* (2015) observed 78% reduction in EPG on day seven and 98% reduction on day 14th followed by 100% reduction by day 28th in neem leaf treated group. When garlic juice (1 g/kg/day) was provided, 11.84% reduction in *Strongyloidea* eggs and 74.25% reduction in *Strongyloidea* larvae respectively, was observed (Batatinha *et al.* 2004). By providing neem leaves powder @ 1.0 g/kg. b.wt, reduction of 21.9% and

34% on day seven and 15th post-treatment respectively was observed (Dongre *et al.* 2015). When neem powder @ 200 mg/kg live weight of the animal was given, reduction of 95.81% in EPG on 28th day was observed (Sarker *et al.* 2016). These studies showed the efficiency of neem and garlic as anthelmintics. However, our herbal treatment did not achieve 100% reduction, which might be due to the ratio combination of garlic with neem.

Changes in the live body weights (kg), DMI (kg/day) and feed efficiency (FE) of the cattle were recorded during the first fortnight period (as initial BW) and last fortnight (as final BW) (Table 3). No significant ($P > 0.05$) difference was found among the treatments which might be due to the fact that cows were mature enough and were in 1st to 3rd stage of lactation. So, the body weight did not increase to a greater extent. But the moderately high values were noticed, although non-significant, in body weight of anthelmintic treated groups compared to the control group. Nematodes have been reported to cause severe damages to GIT of the host, therefore, animal spend their energy and protein for repairing these damages caused by these parasites other than for growth (Khan *et al.* 1988). This might be responsible for the insignificant changes in live animal's body weights in spite of anthelmintic treatment. Another possible reason could be the confinement of the animals to dry commercial feeds as well as leakage of blood and plasma protein to the gastrointestinal tract by the parasites (Egual *et al.* 2007). However, varied reports are available regarding the effect of deworming on body weights as an increase in live weight of sheep while using neem seeds as treatment against nematodes has been reported (Ahmed *et al.* 1994). When pulverized neem seeds at the rate of 100 mg/kg body weight were provided for 60 days, an increase of 1.8% was observed in body weight (Asaduzzaman 1998). There was no significant difference for the DMI in the different groups. However, the analysis showed a significant difference in overall values of DMI, which were significantly ($P < 0.05$) higher in T2 and T4 treatment groups compared to T1 and T3 groups. The FE was better in all anthelmintic treated groups compared to control group; however, it was significantly ($P < 0.05$) better in herbal treated groups (T3 and T4) compared to the synthetic anthelmintic treated group (T2). Neem leaves and seed

Table 3. Mean $\pm$ SE of body weight (kg), dry matter intake (kg/day) and feed efficiency

Parameter		T1 (control)	T2 (synthetic)	T3 (single dose)	T4 (double dose)
Body weight (kg)	Initial	365.71 $\pm$ 7.30	380.57 $\pm$ 9.25	367.57 $\pm$ 12.00	375.20 $\pm$ 18.70
	Final	382.14 $\pm$ 13.73	406.43 $\pm$ 13.43	390.86 $\pm$ 30.26	371.29 $\pm$ 18.49
	Overall	359.53 $\pm$ 3.51	378.33 $\pm$ 3.64	363.62 $\pm$ 4.65	368.34 $\pm$ 5.75
Dry matter intake (kg/day)	Initial	9.52 $\pm$ 0.56	9.79 $\pm$ 0.56	9.08 $\pm$ 0.36	9.43 $\pm$ 0.57
	Final	11.44 $\pm$ 0.85	13.29 $\pm$ 0.90	11.79 $\pm$ 0.40	13.60 $\pm$ 0.62
	Overall	10.49 ^w $\pm$ 0.33	11.95 ^s $\pm$ 0.30	10.71 ^w $\pm$ 0.18	12.21 ^s $\pm$ 0.28
Feed efficiency	Initial	1.06 $\pm$ 0.12	0.97 $\pm$ 0.17	0.97 $\pm$ 0.22	0.99 $\pm$ 0.10
	Final	0.70 ^a $\pm$ 0.13	0.91 ^b $\pm$ 0.07	1.01 ^b $\pm$ 0.10	1.11 ^b $\pm$ 0.08
	Overall	1.03 ^w $\pm$ 0.05	1.11 ^s $\pm$ 0.04	1.14 ^s $\pm$ 0.05	1.25 ^s $\pm$ 0.05

Means bearing different superscript in a row differ significantly ($P < 0.05$).

Table 4. Mean±SE of body condition score of cows in different treatment groups

Fortnights	T1 (control)	T2 (synthetic)	T3 (single dose)	T4 (double dose)
0	3.34±0.07	3.32±0.18	3.25±0.05	3.33±0.13
1	3.04±0.11	3.21±0.09	3.14±0.07	3.11±0.09
2	3.00±0.09	3.14±0.07	3.11±0.05	2.93±0.12
3	2.93±0.12	3.07±0.15	3.04±0.11	2.75±0.16
4	2.89±0.11	2.86±0.13	2.82±0.12	2.82±0.12
5	2.71 ^a ±0.10	2.93 ^{ab} ±0.07	2.86 ^{ab} ±0.09	3.04 ^b ±0.04
6	2.71 ^a ±0.10	2.86 ^{ab} ±0.09	2.96 ^{ab} ±0.09	3.11 ^b ±0.07
7	2.64 ^a ±0.09	2.96 ^{ab} ±0.09	3.04 ^{ab} ±0.10	3.11 ^b ±0.05
8	2.7 ^a ±0.15	3.17 ^{ab} ±0.14	3.07 ^{ab} ±0.13	3.36 ^b ±0.09
Overall	2.87 ^w ±0.04	3.06 ^x ±0.04	3.03 ^x ±0.03	3.08 ^x ±0.04

Means bearing different superscript in a row differ significantly (P<0.05).

extracts as natural dewormers caused a significant increase of body weight in sheep (Amin *et al.* 2009). Significant higher overall DMI might be due to better reduction in the worm load. Analogous findings were reported by previous studies (Radhakrishnan *et al.* 2007, Yang *et al.* 2007) where they found higher DMI in the neem supplemented and garlic supplemented groups compared to the control diet, respectively. The better FE in herbal treated groups, could be due to the phytochemical activity of condensed tannin (CT) in herbs which has beneficial effects by forming protein-tannin complex, decreasing availability of feed protein for ruminal degradation and ammonia nitrogen release (Makkar *et al.* 2003). Earlier studies showed that CT and saponin had variable effects on ruminal VFA concentration. The mean values of BCS of control and anthelmintic treated groups for eight fortnight period is presented in Table 4. No significant difference was found in different treatment groups for BCS up to fourth fortnight intervals after the treatment. In the later fortnight period (i.e. fifth to eighth), the BCS was found significantly (P<0.05) higher in T4 group compared to control group. However, T2 and T4 group did not differ significantly from control and T4 treatment group. The effect of BCS between groups after fifth fortnight onwards might be due to improved utilization of nutrients after removal of the worm burden from the GIT. DMI is not much affected but the improvement in BCS might be due to better utilization of the feed. The herbal extract mixture treated groups could have benefitted from better utilization of proteins in the presence of tannins resulting into better nutrient utilization along with lower worm load. The present study concluded that garlic and neem combination as herbal anthelmintic (1:5 ratio) in double dose is as effective as the synthetic dewormer in controlling the nematode parasites. Besides their anthelmintic potency, garlic and neem also improved overall body condition and feed efficiency of lactating cows.

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SUMMARY

This study was conducted to evaluate the anthelmintic efficacy of neem and garlic combination as an effective herbal anthelmintic alternative for chemical dewormers in dairy cows. For this, EPG count for GI parasites, body weight and BCS of cows were studied. Freshly calved crossbred KF (n=28) cows were selected and divided into 4 groups (T1, T2, T3, T4), each having 7 cows. The cows of T1 were used as control; T2, cows were provided the commercial (synthetic) dewormer; T3, cows were provided with a dose of combination of garlic and neem in the ratio of 1:5 @ 4 g/kg body wt. at 7 days after parturition and T4, in addition to first dose at 7 days, a second dose was also supplemented at day 60. Sampling of faecal samples was done on 0, 1, 2, 3, 4, 5, 6, 7, 8 fortnights relative to the deworming treatment. The EPG counting was done by using McMaster counting chamber. The average fecal egg count (FCE) reduction was significantly more in T2 (83.20%) group followed by T4 (77.48%). The BCS and FCE were significantly (P<0.05) better in all the anthelmintic treated groups compared to control group. However, the FCE was significantly (P<0.05) better in herbal treated groups (T3 and T4) compared to the synthetic anthelmintic treated group (T2). Based on these findings, it can be concluded that garlic and neem combination is effective herbal dewormer/anthelmintic and might be used against nematodes in dairy cattle as an effective alternative of synthetic anthelmintics drugs.

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